Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 3-10, 13, and 24-37 are pending in the application, with claim 1, 8, 9, 10 and 31 being the independent claims. Claims 2, 11, 12, and 14-23 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 24-37 are sought to be added. Support for amendments to claim 1 may be found, *inter alia*, at page 18, lines 11-27 of the specification. Support for amendments to claims 8-10 may be found, *inter alia*, at page 21, lines 7-25 of the specification. Support for amendments to claim 13 may be found, *inter alia*, at page 34, line 27, through page 35, line 6 of the specification. Support for new claim 24 may be found, *inter alia*, at page 12, lines 25-28, and at page 34, lines 3-8 of the specification. Support for new claims 25-37 may be found, *inter alia*, at page 18, lines 11-27 of the specification. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Priority

The Examiner states that Applicants are not entitled to the benefit of priority for claims directed to SEQ ID NOS:1-3 because the sequences, as amended, do not appear in the priority applications.

It is not believed that the amendments to the Sequence Listing introduce new matter since they reflect the correct nucleotide and amino acid sequences contained in the deposited clones, ATCC Deposit Nos. 209933, 209934 and 98809. The clones contained in ATCC Deposit Nos. 209933 and 209934 were deposited on June 16, 1998 (prior to the filing of the first provisional application) and the clone contained in ATCC Deposit No. 98809 was deposited on July 10, 1998 (prior to the filing of the second provisional application). See the specification at page 16, lines 7-10. Therefore, since the correct sequences were inherent to the deposited clones at the time the application was filed, and the deposit information formed part of the original specification, we believe that no new matter was introduced by the amendment to correct sequence errors. Accordingly, it is believed that Applicants are entitled to the June 25, 1998 filing date for sequences contained within ATCC Deposit Nos. 209933 and 209934 and the July 24, 1998 filing date for the sequence contained within ATCC Deposit No. 98809.

Drawings

The Draftsperson has objected to the drawings. Formal drawings which are believed to correct the defects as noted in the Draftsperson's report are attached hereto.

Objection to the Specification

The Examiner has objected to the Brief Description of Drawings section because the application does not refer to the multiple panels of Figures 1A-D, 3A-B, and 8A-E. Applicants have amended the specification to bring it into conformity with the figures. Accordingly, Applicants respectfully request that the Examiner withdraw the objection.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 1 and 3-10 under 35 U.S.C. § 112, first paragraph, as allegedly being non-enabled. Specifically, the Examiner contends that the variants of claim 1(e) and subfragments specified in claims 8(a), 9(a) and 10(a) are non-enabled because the specification does not provide any guidance how to make the divergent sequences and the polypeptide may not possess the activity proposed in the specification. The Examiner asserts that the predictability of changes to the amino acid sequence is practically nil as far as the biological activity is concerned. Applicants have amended claims 1, 8, 9 and 10. Applicants respectfully traverse this rejection as it may apply to the pending claims.

In order for a claim to be enabled, the specification must teach one of ordinary skill in the art how to make and use the invention without undue experimentation. The factors that can be considered in determining whether an amount of experimentation is undue have been set forth in *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). Among these factors are: the amount of effort involved, the guidance provided by the specification, the presence of working examples, the amount of pertinent literature and the level of skill in the art. The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine. *See id.*

While the predictability of the art can be considered in determining whether an amount of experimentation is undue, mere unpredictability of the result of the experiment is not a consideration. Indeed, in *In re Angstadt*, the Court of Custom and Patent Appeals has specifically cautioned that the unpredictability of the result of an experiment is not a basis to conclude that the amount of experimentation is undue:

> If to fulfill the requirements of 112, first paragraph, an applicant's disclosure must provide guidance which will enable one skilled in the art to determine, with reasonable certainty before performing the reaction whether the claimed product will be obtained, . . . then all "experimentation" is "undue" since the term "experimentation" implies that the success of the particular activity is uncertain. Such a proposition is contrary to the basic policy of the Patent Act.

537 F.2d at 503, 190 U.S.P.Q. at 219 (emphasis in the original).

Amended claim 1 is drawn to polynucleotide sequences encoding mouse and human Dnmt3a and Dnmt3b polypeptides and polynucleotides at least 95% identical to said polynucleotide sequences.

Applicants assert that the specification is enabling for sequences at least 95% identical, such that the resultant activity of the polypeptide is not unpredictable, as the Examiner alleges. The Applicants respectfully direct the Examiner's attention to Example 1 which describes, *inter alia*, Applicants' cloning of mouse and human *de novo* cytosine methyltransferase genes and Applicants' extensive sequence analysis thereof.

FIGS. 3A and 3B demonstrate sequence conservation between Dnmt3a and Dnmt3b. For example, Dnmt3a and Dnmt3b exhibit 51% overall amino acid identity and 76% identity in the catalytic domain of the proteins (see page 54, lines 9-13 of the specification). Using the sequence alignments as a guide, the skilled artisan would be able to make predictions about which amino acids may be mutated for the protein to retain biological activity. For

example, if at a particular locus Dnmt3a encodes a phenylalanine and Dnmt3b encodes tryptophan, the artisan would predict that Dnmt3a may be mutated to tryptophan at that locus and still retain biological activity.

Moreover, Applicants demonstrate that Dnmt3a and Dnmt3b also exhibit similarity to other methyltransferase polypeptides. Figure 5A presents sequence alignment of the methyltransferase catalytic domains. As the catalytic domains are able to methylate the cytosine on DNA, the artisan could make predictions about a consensus sequence and make conservative mutations based on this sequence. Applicants have also aligned the cysteine rich region of Dnmt3a and Dnmt3b with related homologous sequences. Using this information, the artisan could make predictions when making substitutions to the sequence.

Applicants have also conducted a phylogenetic analysis of the Dnmt3 proteins with known methyltransferases. Applicants note that Dnmt3a and Dnmt3b are most closely related to a bacterial DNA methyltransferase, and are more distantly related to eukaryotic Dnmt1 (see page 58, lines 1-10 of the specification). Applicants assert that knowledge of the phylogenetic relationships of Dnmt3a and Dnmt3b with other known methyltransferases provide useful information to the artisan regarding which mutations are likely to maintain biological activity.

In making the rejection, the Examiner has relied on a publication by Lazar *et al.*, *Mol. Cell Biol.* 8(3):1247-1252 (1988). Lazar mutated conserved residues in TGF-α and then tested the activity of mutants. Lazar describes the rationale for mutagenesis at specific residues within TGF-α:

> Figure 1 shows the amino acid sequence of TGF-α in which the residues are conserved among all the EGF-like peptides described thus far (EGF, TGF-α, and EGF-like viral proteins) are enclosed in bold circles. . . .We concentrated on the two

> *conserved* amino acids in the carboxyl terminus, Asp-47 and Leu-48. The Asp in position 47 is conserved among the EGFs and TGF-α (human or murine), but not among the EGF-like viral proteins (vaccinia growth factor, Shobe fibroma growth factor, or myoma growth factor), whereas Leu 48 is conserved among all the EGF-like peptides so far described. . . .We designed a series of mutations in these two positions.

Lazar *et al.* at 1248 (emphasis added). Applicants assert that a skilled artisan would anticipate that mutation of conserved residues would likely disrupt biological activity. Indeed, Lazar *et al.* observed diminished biological effects for some of the mutants (*Id.*, at Table 1). The approach of Lazar *et al.* was to dissociate interactions responsible for TGF-α binding to it's receptor from those interactions involved in signal transduction, with the goal of creating antagonists of the TGF-α receptor (see Introduction).

Such an approach contrasts sharply with that of a potential infringer of Applicants' claims that is motivated to make substitutions to Applicants' sequence to obtain sequences that are at least 95% identical that maintained *de novo* methyl transferase activity. In this regard, a potential infringer would not be motivated to mutate *conserved* residues, like that shown by Lazar *et al.*, but would rather mutate *nonconserved* residues. Using Applicants' sequence alignments as a guide, a potential infringer would mutate the nonconserved residues to the corresponding residues found in the homologous proteins to achieve the highest probability that the mutants maintain biological activity. The strong sequence similarity found in Dnmt3a and Dnmt3b lends itself quite readily to such an approach. Therefore, Applicants deem it necessary that the claims encompass 95% nucleotide identity to guard against this possibility. Moreover, Applicants have added a new dependent claim 25 that requires that the nucleic acid molecule of claim 1 encode a polypeptide capable of methylating cytosine in DNA.

On the other hand, if a potential infringer were interested in making mutations that abrogated biological activity of the *de novo* methyltransferases, Applicants sequence alignments provide guidance on which of the residues would be most appropriate to mutate. For example, a potential infringer would be motivated to mutate conserved residues and test for altered activity. Applicants assert that such experimentation would be routine, in view of Applicants' extensive sequence analysis. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Claims 8(a), 9(a), and 10(a) are drawn to isolated nucleic acid molecules consisting of at least 50, 30 and 100 contiguous nucleotides of SED ID NOS:1, 2 and 3, respectively. The Examiner alleges that subfragments may not encode polypeptides that are capable of acting as enzymes which methylate DNA.

Applicants assert that the claim does not require that the polynucleotides encode proteins or fragments capable of methylating DNA. In contrast, the specification discloses that such fragments may be useful as probes or primers for screening or amplifying Dnmt3. Polynucleotide fragments can also be used for making antisense oligonucleotides to inhibit Dnmt3 expression. Therefore, Applicants assert that the claims are enabled. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claim 2 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for recitation of the phrase "under stringent conditions." Applicants have canceled claim 2 without prejudice or disclaimer. Accordingly, this rejection is now moot.

The Examiner rejected claims 8 and 9 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for recitation of "subfragment." Applicants respectfully traverse this rejection as it may be applied to the pending claims.

Applicants respectfully disagree with the Examiner's assertion that subfragment is indefinite for not setting forth the metes and bounds of the claim. Applicants claim a polynulceotide of at least 50 or 30 continguous nucleotides provided that the nucleotides are *not* certain Genbank sequences or subfragments thereof as set forth in the claim. Thus, any particular subfragment is bounded by the 1) 50 or 30 contiguous nucleotide requirement of the claim and 2) the length of the sequence specified by the Genbank Accession Number. Accordingly, Applicants assert that the claim is definite to one skilled in the art, and respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner has rejected claim 13 for recitation of "effective amount," alleging that it is not clear what amount of the *de novo* cytosine methyltransferase polypeptide is deemed effective in order to methylate DNA. Applicants respectfully traverse the rejection as it may be applied to amended claim 13.

Applicants have amended claim 13 by deleting the phrase "effective amount." Applicants assert that the specification provides ample guidance to those skilled in the relevant art to perform *in vitro* methylation reactions with polypeptides of the invention. Guidance may be found, *inter alia*, at page 64, line 18, through page 66, line 4. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Rejections under 35 U.S.C. § 102

At page 3 of the Office Action, the Examiner alleged that a date of priority for original claims 1-10 is the instant application's filing date of July 13, 2001, as sequences identical to SEQ ID NOS:1-3 as amended are not found in the priority documents. Based on the alleged priority date of July 13, 2001, the Examiner rejected claims 1-4 under 35 U.S.C. § 102(b) as allegedly anticipated by Okano *et al.* and Xie *et al.* Applicants have amended claim 1 and have canceled claim 2. Applicants respectfully traverse the rejection as it may be applied to the pending claims.

Applicants respectfully disagree with the Examiner that the instant application's priority date in regard to SEQ ID NOS:1-3 is July 13, 2001. Since the correct nucleic acid sequences were inherent to the deposited clones at the time the application was filed, and the deposit information formed part of the original specification, we believe that no new matter was introduced by the amendment filed on July 23, 2001. In this regard, the specification states: "While the ATCC deposits are believed to contain the *de novo* DNA cytosine methyltransferase cDNA sequences shown in SEQ ID NOs:1, 2, 3, and 4, the nucleotide sequences of the polynucleotide contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein." See page 16, lines 12-17 of the specification. Therefore, Applicants are entitled to the June 25, 1998 filing date for sequences contained within ATCC Deposit Nos. 209933 and 209934 and the July 24, 1998 filing date for the sequences contained within ATCC Deposit No. 98809 (see above paragraph entitled "Priority"). Accordingly, Applicants respectfully request that the

Examiner reconsider and withdraw the rejection as Okano *et al.* and Xie *et al.* are not prior art under 35 U.S.C. § 102(b) to the present claims.

The Examiner also rejected claims 2 and 9 under 35 U.S.C. § 102(e) as allegedly anticipated by SEQ ID NO:47 contained in U.S. Patent No. 6,183,968. Applicants have amended claim 9 and have canceled claim 2. Applicants respectfully traverse the rejection as it may be applied to the pending claims.

Applicants have amended claim 9 to recite that the isolated nucleic acid molecule comprises at least 30 contiguous nucleotides of SEQ ID NO:2. Applicants submit that U.S. Patent No. 6,183,968 does not disclose polynucleotides that meet this limitation. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

The Examiner also rejected claims 8 and 10 under 35 U.S.C. § 102(b) as allegedly being anticipated by the nucleic acid sequence (Accession No. AAT21884) found in document WO95142772. Applicants have amended claims 8 and 10. Applicants respectfully traverse the rejection as it may be applied to the pending claims.

Applicants have amended claim 8 to recite that the isolated nucleic acid molecule comprises at least 50 contiguous nucleotides of SEQ ID NO:1. Applicants have amended claim 10 to recite that the isolated nucleic acid molecule comprises at least 100 contiguous nucleotides of SEQ ID NO:3. Applicants submit that the document WO95142772 does not disclose polynucleotides that meet these limitations. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Finally, the Examiner has rejected claim 13 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 6,492,168. Applicants have amended claim 13. Applicants respectfully traverse the rejection as it may be applied to the pending claim.

Applicants have amended claim 13 to require that the *de novo* cytosine methyl transferase polypeptide be encoded by a polynucleotide contained in ATCC Deposit Nos. 209933, 209934, 98809 or 326637. Applicants submit that U.S. Patent No. 6,492,168 does not disclose the *de novo* cytosine methyl transferase polynucleotides of claim 1. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Rejections under 35 U.S.C. § 103

The Examiner rejected claims 1 and 3-7 under 35 U.S.C. § 103(a) as being unpatentable over Okano *et al.* as evidenced by Accession No. AF068625 and Xie *et al.* as evidenced by Accession No. AF067972, in view of Ausubel *et al.* Applicants have amended claim 1. Applicants respectfully traverse the rejection as it may be applied to the pending claims.

Okano *et al.* relates to polypeptides indicated by Applicants' SEQ ID NOS:5 and 6, that are encoded by the polynucleotides contained within the ATCC Deposit Nos. 209933 and 209934. As stated *supra*, Applicants are entitled to the June 25, 1998 filing date for sequences contained within ATCC Deposit Nos. 209933 and 209934. Thus, Okano *et al.* is not prior art to the parts (a) and (b) of claim 1.

Xie *et al.* relates to polypeptides indicated by Applicants' SEQ ID NOS:7 and 8. SEQ ID NO:7 is contained within the ATCC Deposit No. 98809. As stated *supra*,

Applicants are entitled to the July 24, 1998 filing date for the sequence contained within ATCC Deposit No. 98809. Thus, Xie *et al.* is not prior art to parts (c) and (d) of claim 1. Therefore, Ausubel *et al.* cannot be combined with Okano *et al.* and Xie *et al.*to make a proper rejection under 35 U.S.C. § 103(a). Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Specification

At page 4, the paragraph starting at line 10 was replaced with the following paragraph:

Figures [1A-1D] 1A-1 through 1A-4 show[s] the nucleotide sequence[s] of the mouse Dnmt3a gene. Figures 1B-1 through 1B-4 show the nucleotide sequence of the mouse Dnmt3b gene. Figures 1C-1 through 1C-4 show the nucleotide sequence of the human DNMT3A gene. Figures 1D-1 through 1D-4 show the nucleotide sequence of the human DNMT3B gene. [and Dnmt3b and human DNMT3A and DNMT3B genes, respectively.]

At page 4, the paragraph starting at line 15 was replaced with the following paragraph:

Figures 3A-1 and 3A-2 show[s] a comparison of mouse Dnmt3a and Dnmt3b amino acid sequences, and Figures 3B-1 and 3B-2 present[s] a comparison of the protein sequences of human DNMT3A and DNMT3B1.

At page 5, the paragraph starting at line 5 was replaced with the following paragraph:

Figures 8A-E demonstrate[s] *in vitro* analysis of *de novo* and maintenance activities of Dnmt3a, Dnmt3b1 and Dnmt3b2 proteins.

In the Claims

Please substitute the following claim 1 for the pending claim 1:

1. (once amended) An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of:

a. a polynucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 908 in SEQ ID NO:5;

b. a polynucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 859 in SEQ ID NO:6;

c. a polynucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 912 in SEQ ID NO:7;

d. a polynucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 853 in SEQ ID NO:8; [and]

e. a polynucleotide sequence that is at least [90%] 95% identical to the polynucleotide sequence of (a), (b), (c) or (d); and

f. a polynucleotyide sequence complementary to the polynulceotide sequence of (a), (b), (c), (d) or (e).

Please substitute the following claim 8 for the pending claim 8:

8. (once amended) An isolated nucleic acid molecule comprising polynucleotides selected from the group consisting of:

a. at least [20] 50 contiguous nucleotides of SEQ ID NO:1, provided that said nucleotides are not AA052791(SEQ ID NO: 9); AA111043(SEQ ID NO:10); AA154890(SEQ ID NO:11); AA240794(SEQ ID NO:12); AA756653(SEQ ID NO:13); W58898(SEQ ID NO:14); W59299(SEQ ID NO:15); W91664(SEQ ID NO:16); W91665(SEQ ID NO:17); or any subfragment thereof; and

b. a nucleotide sequence complementary to a nucleotide sequence in (a).

Please substitute the following claim 9 for the pending claim 9:

9. (once amended) An isolated nucleic acid molecule comprising polynucleotides selected from the group consisting of:

a. at least [20] 30 contiguous nucleotides of SEQ ID NO:2, provided that said nucleotides are not AA116694 (SEQ ID NO:18); AA119979 (SEQ ID NO:19); AA177277 (SEQ ID NO:20); AA210568 (SEQ ID NO:21); AA399749 (SEQ ID NO:22); AA407106 (SEQ ID NO:23); AA575617 (SEQ ID NO:24); or any subfragment thereof; and

b. a nucleotide sequence complementary to a nucleotide sequence in (a).

Please substitute the following claim 10 for the pending claim 10:

10. (once amended) An isolated nucleic acid molecule comprising polynucleotides selected from the group consisting of:

a. at least [20] 100 contiguous nucleotides of SEQ ID NO:3, provided that said nucleotides are not AA004310 (SEQ ID NO:25); AA004399 (SEQ ID NO:26); AA312013 (SEQ ID NO:27); AA355824 (SEQ ID NO:28); AA533619 (SEQ ID NO:29); AA361360 (SEQ ID NO:30); AA364876 (SEQ ID NO:31); AA503090 (SEQ ID NO:32); AA533619 (SEQ ID NO:33); AA706672 (SEQ ID NO:34); AA774277 (SEQ ID NO:35); AA780277 (SEQ ID NO:36); H03349 (SEQ ID NO:37); H04031 (SEQ ID NO:38); H53133 (SEQ ID NO:39); H53239 (SEQ ID NO:40); H64669 (SEQ ID NO:41); N26002 (SEQ ID NO:42); N52936 (SEQ ID NO:43); N88352 (SEQ ID NO:44); N89594 (SEQ ID NO:45); R19795 (SEQ ID NO:46); R47511 (SEQ ID NO:47); T50235 (SEQ ID NO:48); T78023 (SEQ ID NO:49); T78186 (SEQ ID NO:50); W22886 (SEQ ID NO:51); W67657 (SEQ ID NO:52); W68094 (SEQ ID NO:53); W76111 (SEQ ID NO:54); Z38299 (SEQ ID

NO:55); Z42012 (SEQ ID NO:56); G06200(SEQ ID NO:74); or any subfragment thereof; and

b. a nucleotide sequence complementary to a nucleotide sequence in (a).

Please substitute the following claim 13 for the pending claim 13:

13. (once amended) A method for *in vitro de novo* methylation of DNA, comprising:

a. contacting said DNA with [an effective amount of] a *de novo* DNA cytosine methyltransferase polypeptide encoded by the nucleic acid molecule of claim 1;

b. providing an appropriately buffered solution with substrate and cofactor; and

c. purifying said DNA.

Claims 2, 11, 12, and 14-23 were canceled.

New claims 24-37 were added.